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Cytokine Production by CAPAN-1 and CAPAN-2 Cell Lines

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Recently, there has been a great deal of interest in the role of cytokines in acute pancreatitis. Serum levels of IL-1, IL-6, and TNF-α have been demonstrated to be elevated in acute pancreatitis. We hypothesized that cytokines may be produced primarily by pancreatic parenchymal cells. Reasoning that ductal epithelium is the cell type most likely to be exposed to noxious stimuli in common causes of pancreatitis, such as ERCP and passage of a gallstone, we examined the response of well differentiated pancreatic ductal adenocarcinoma cell lines to stimuli known to stimulate cytokine production in other cells. CAPAN-1 and CAPAN-2 cells were incubated with endotoxin or TNF-α. The supernatant was assayed for production of IL-1, IL-6, and IL-8 by ELISA. The cells were assayed for activation of the transcription factor NF-κB by electrophoretic mobility shift assay. There was no detectable production of IL-1 by either cell line. CAPAN-1 cells had concentration-dependent production of IL-6 and IL-8 in response to both endotoxin and TNF-α. CAPAN-2 cells had concentration-dependent production of IL-6 and IL-8 in response to TNF-α. They had low level expression of IL-8 that was unaffected by any concentration of LPS, and no detectable production of IL-6 in response to LPS. These findings suggest that pancreatic duct cells may take an active part in the pathogenesis of acute pancreatitis through the production of cytokines.

KEY WORDS: ductal epithelium; cytokines; tumor necrosis factor-α; interleukin-1; interleukin-6; interleukin-8.

Recently, several investigators have developed an interest in the role of cytokines in acute pancreatitis. Cytokines are low-molecular-weight polypeptides (generally <30 kDa) synthesized and released by most nucleated cells including monocytes and macrophages (1–11). Multiple factors stimulate cytokine production including fungi, viruses, irradiation, endotoxin [lipopolysaccharide (LPS)] and reactive oxygen intermediates (ROIs). Cytokines generally fall into two groups: (1) those that primarily act as growth factors such as epidermal growth factor (EGF) and IL-6, and (2) those that primarily possess inflammatory properties such as interleukin-1 (IL-1), IL-8, and tumor necrosis factor (TNF). Growth factor cytokines often secondarily possess inflammatory properties and *vice versa*. Each cytokine has its own distinct amino acid sequence, structure, and high-affinity cell surface receptor(s). Cytokines of particular interest to our laboratory in relation to pancreatitis (IL-6, TNF, and IL-8) have numerous and often overlapping diverse biologic activities (4–11). Importantly, many of the biologic activities of cytokines, such as fever, hypermetabolism, anorexia neutrophilia, increased endothelial permeability, and lung edema, are also clinical manifestations or complications of pancreatitis.

Because there is a growing body of literature demonstrating elevated levels of cytokines in human pancreatitis (12–19), we hypothesized that cytokines might be primarily involved in the pathogenesis of

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acute pancreatitis. We further hypothesized that pancreatic parenchymal cells might be the source of inflammatory cytokines. In two clinical situations associated with human pancreatitis, passage of gallstones and endoscopic retrograde pancreatography, the pancreatic duct would appear to be most exposed to noxious stimuli. We therefore chose to investigate first the production of cytokines by pancreatic duct cells.

Since primary cultures of normal pancreatic ductal epithelial cells are difficult to obtain in quantities sufficient to perform biochemical and cell biological studies, we elected to use two well-differentiated pancreatic carcinoma cell lines, CAPAN-1 and CAPAN-2, as surrogates. The use of well-differentiated tumor cell lines for preliminary investigation of the tissue of interest is a well-established practice. Caco-2 cells have been used to study the normal responses of colonic epithelium (20, 21), and Hep G-2 cells have been used to study the cell physiology of normal hepatocytes (22, 23). The CAPAN cell lines have been used to study other aspects of pancreatic function (24, 25).

MATERIALS AND METHODS

Reagents and Cells. The CAPAN-1 and CAPAN-2 cell lines, pancreatic adenocarcinoma cell lines that retain good ductal differentiation and biochemical markers, were obtained from the American Type Culture Collection. IL-1, IL-6, and IL-8 ELISA kits were obtained from R&D Systems (Minneapolis, Minnesota). Lipopolysaccharide (endotoxin) was obtained from Sigma. Tumor necrosis factor-α (TNF-α) was a generous gift from Knoll Laboratories. Polyclonal antibodies to p65 (Rel A) and p50 were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, California). All other chemicals and reagents were tissue culture grade and were obtained from commercial sources.

Cell Culture. CAPAN-1 and CAPAN-2 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 μg/ml streptomycin, penicillin, and 2 mM glutamine. Subconfluent cultures of cells were incubated with varying concentrations of TNF-α or LPS for 24 hr.

IL-1, IL-6, and IL-8 ELISA. Cell cultures were aspirated and centrifuged. The conditioned medium supernatant was assayed for IL-1, IL-6, and IL-8 using commercially available ELISA kits from R&D Systems, according to the manufacturer's directions.

Electrophoretic Mobility Shift Assay. The assay for NF-κB was performed as described by Sen and Baltimore (26). Nuclear extracts of the cell cultures were prepared according to the method if Dignam et al (27). ^{32}P-end-labeled nucleotide probes containing the κB enhancer DNA element were prepared according to the manufacturer's protocols. Incubation of nuclear extracts with the probe for 20 min at room temperature was carried out in the presence of nonspecific competitor DNAs. Following binding, the complexed and uncomplexed DNA in the mixture was resolved by electrophoresis on a 6% low-ionic-strength, nondenaturing polyacrylamide gel. Visualization was by autoradiography. Specificity was verified by the addition of a 100-fold excess of unlabeled nucleotide as a competitor.

Supershift Assay. Supershift assays were performed with polyclonal antibodies to the NF-κB proteins p65 (Rel A) and p50. These antibodies were used in the supershift assays at a concentration of 1 μg/25 μl, and the samples were incubated at 25°C for 15 min prior to the addition of the ^{32}P-labeled NF-κB oligonucleotide probe.

RESULTS

Interleukin 1. We were not able to detect any production of IL-1 by either cell type after incubation of the cells with either LPS or TNF-α.

Interleukin 6. There was a basal production of IL-6 by CAPAN-1 cells that was significantly augmented by both LPS and TNF-α in a concentration-dependent fashion (Figure 1a and b). Untreated cells produced a mean of 90 pg/ml of IL-6 at 24 hr, while 10 μg/ml of LPS produced a mean of 195 pg/ml, and 2000 units/ml of TNF-α produced a mean of 180 pg/ml of IL-6. There was no detectable production of IL-6 by CAPAN-2 cells in response to LPS (Figure 1a). Incubation of CAPAN-2 cells with TNF-α produced a minimal, but still concentration-dependent, amount of IL-6 (Figure 1b), reaching a mean of 45 pg/ml of IL-6 at 24 hr in response to 2000 units/ml of TNF-α.

Interleukin 8. Both CAPAN-1 and CAPAN-2 cell lines demonstrated basal production of IL-8. The CAPAN-1 cultures produced a mean of 2000 pg/ml, and the CAPAN-2 cells produced a mean of 400 pg/ml of IL-8 in the basal state. Incubation with LPS produced a concentration-dependent increase in IL-8 production only in the CAPAN-1 cells, rising from a mean of 2000 pg/ml to 2800 pg/ml at 24 hr. LPS did not result in a significant change in the production of IL-8 by the CAPAN-2 cells (Figure 2a). Incubation with TNF-α produced a concentration-dependent rise in IL-8 from both cell lines. TNF-α-stimulated IL-8 concentrations rose to a mean of 1420 pg/ml in the CAPAN-1 cell cultures at 24 hr. In the CAPAN-2 cells, IL-8 concentrations rose to a mean of 1450 pg/ml at 24 hr, at a concentration of 2000 units of TNF-α per milliliter (Figure 2b).

NF-κB. NFκB activity in nuclear extracts from the cell lines showed a pattern of activation parallel to the production of cytokines. There was activation of NF-κB in CAPAN-1 cells in response to both LPS and TNF-α in a concentration-dependent fashion. Binding was completely eliminated by an excess of

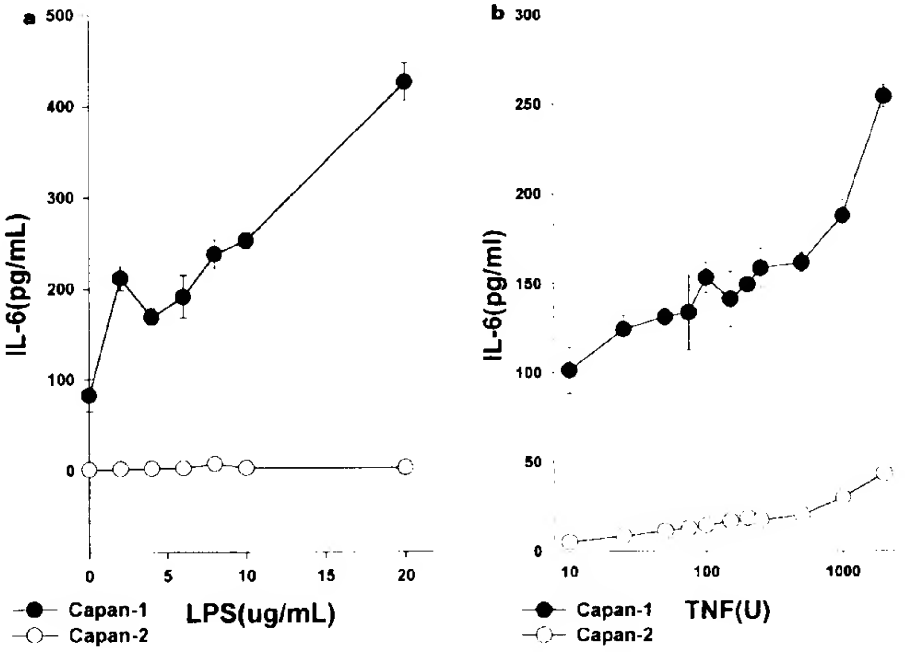

Fig 1. Production of IL-6 by CAPAN-1 and CAPAN-2 cell lines in response to LPS (1a) and TNF (1b).

unlabeled specific nucleotide. Supershift with p50 and p65 antibodies demonstrated two species, one containing p50 and one containing p65, with no apparent presence of p50/p65 heterodimer (Figure 3a). CAPAN-2 cells showed concentration-dependent activation of NF-κB only in response to TNF-α. There was no response to LPS. As with the CAPAN-1 cells, binding was completely eliminated by an excess of

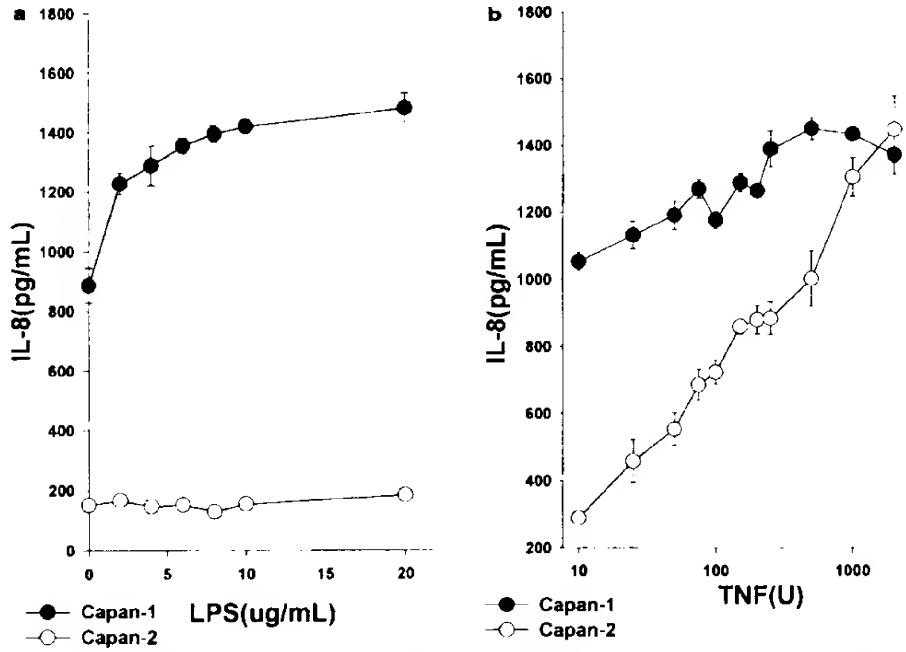

Fig 2. Production of IL-8 by CAPAN-1 and CAPAN-2 cell lines in response to LPS (2a) and TNF (2b).

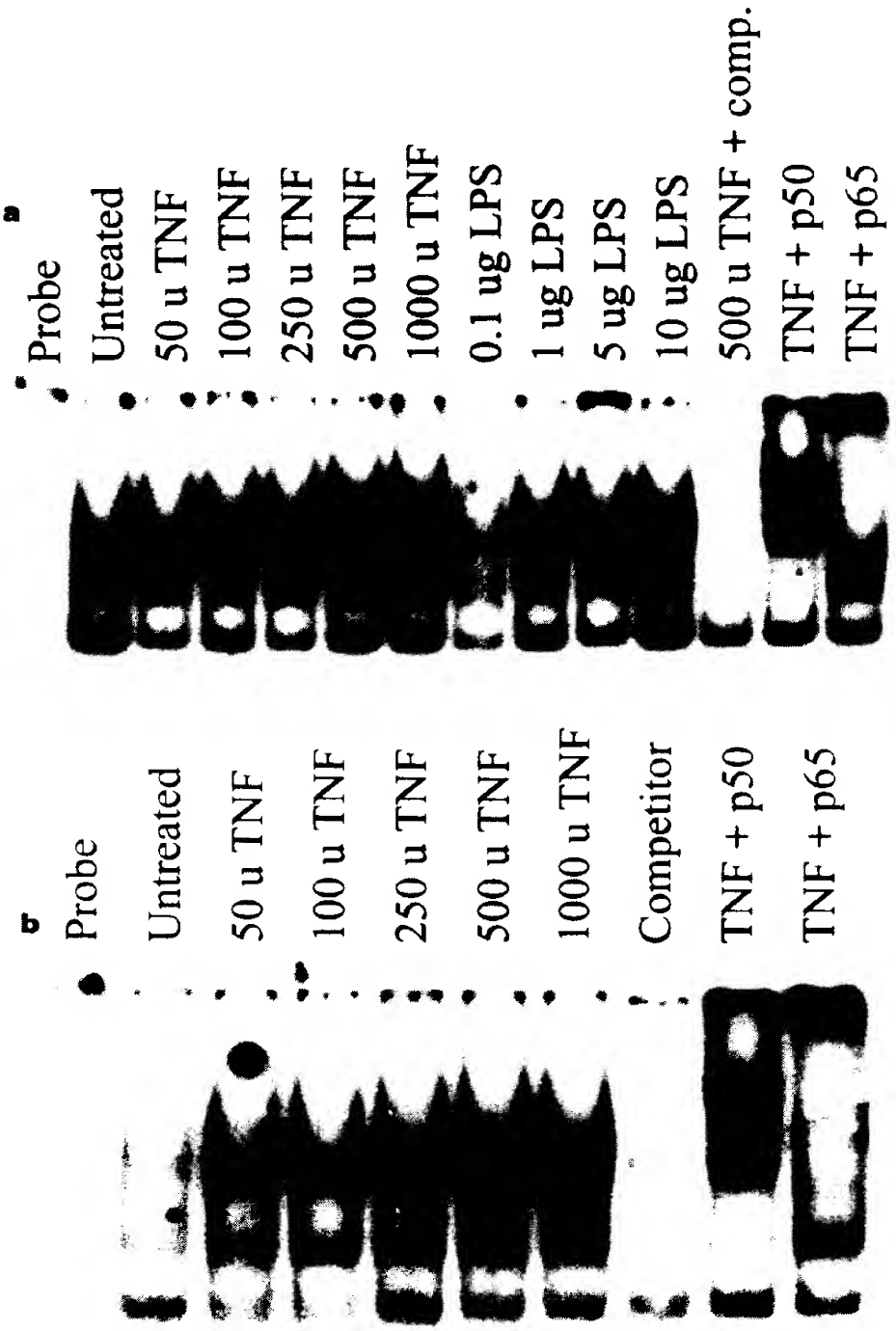

Fig 3. Electrophoretic mobility shift assays and supershift assays of NF-κB activity from CAPAN-1 and CAPAN-2 cell lines. CAPAN-1 cells demonstrate concentration-dependent activation of NF-κB in response to incubation with either TNF or LPS. An excess of unlabeled nucleotide confirms specificity. Antibodies to p50 and p65 show the presence of bands containing either protein, but no heterodimer (3a). CAPAN-2 cells demonstrate concentration-dependent activation of NFκB in response to incubation with TNF alone. Incubation with LPS did not produce activation of NFκB, and is not shown. An excess of unlabeled nucleotide confirms specificity. Antibodies to p50 and p65 show the presence of bands containing either protein, but no heterodimer (3b).

unlabeled specific nucleotide. The same pattern of p50 and p65 species, with an absence of p50/p65 heterodimer was also seen (Figure 3b).

DISCUSSION

Our laboratory is the first to describe the production of cytokines by cells derived from pancreatic ducts. Several investigators have demonstrated elevated levels of cytokines in clinical acute pancreatitis but have viewed them as merely indirect markers of the degree of pancreatic inflammation. The underlying assumption is that the cytokines are derived from infiltrating inflammatory cells and not from the glandular parenchyma itself. Most recently, it has been demonstrated that pancreatic acinar cells produce, release, and respond to TNF-α (28). By demonstrating the production of cytokines by duct cells, we hope to open a new line of investigation. Our hypothesis was that noxious stimuli acting on the pancreatic ducts, such as the influx of endotoxin or TNF into the duct during the passage of an infected or colonized gallstone, would induce the production of inflammatory cytokines by pancreatic duct cells.

Our studies demonstrate that cells derived from pancreatic ductal epithelium are capable of producing two important cytokines, IL-6 and IL-8, in response to insults that would be reasonably expected to occur in clinical settings. These findings offer important new insights into the pathophysiology of acute pancreatitis. Local production of IL-8, a potent neutrophil chemotactic factor, may be the process responsible for the initial infiltration of inflammatory cells in acute pancreatitis. Similarly, local production of IL-6 producing high concentrations in the portal blood may be responsible for the drop in albumin seen in severe acute pancreatitis. Finally, the local production of these and other cytokines may result in the dysfunction or death of endothelial or acinar cells, producing in edema and parenchymal necrosis, the essential features of acute pancreatitis.

The production of these cytokines was accompanied by the activation of a key inflammatory response transcription factor, NF-κB. The pattern of response strongly suggests that this transcription factor is involved in the production of cytokines by these cell lines. Since NF-κB also is a cytokine sensitive to oxidant stress, this observation strengthens the hypothesis that antioxidants may play a role in preventing pancreatitis. Our laboratory is actively pursuing investigations in both *in vitro* and *in vivo* to further develop this promising new area of pancreatic research.

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